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In vitro assessment of crude extract from Gomphrena weed (*Gomphrena celosioides* Mart.) for control of plant pathogenic fungi causing chili diseases

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Abstract The phytochemical screening of both the flowers and other aerial parts of the Gomphrena weed extract (*Gomphrena celosioides* Mart.) presented alkaloids, flavonoids, saponins, phenolics/tannins, and terpenoids. The extracts from both parts at 80, 160, and 240 mg/ml were significantly inhibited the mycelial growth of *C. gloeosporioides* and *Fusarium* sp. compared to inoculated control by paper disc diffusion. The extract from both parts of the Gomphrena weed at the highest concentration (160 mg/ml) significantly showed the greatest inhibitory effect on mycelial growth of *Sclerotium* sp. (100 percent), followed by *C. gloeosporioides* (82.5 percent), *Cercospora* sp. (41.76 percent), and *Fusarium* sp. (24.41 percent) when tested using the poisoned food technique. The spore germination test revealed both parts of the weed extract yielded concentrations which were completely inhibited (100 percent) on the spore germination of *C. capsici*, *C. gloeosporioides*, and *Fusarium* sp.

Keywords: Phytochemical components, Plant extracts, Anthracnose, Frogeye leaf spot, Root wilt

Introduction

Chili (*Capsicum* spp.) is one of the important economic crops in Thailand. It enjoys great popularity as a major culinary ingredient and export product (Montri *et al*., 2009). In 2020, production of this crop was approximately 18,000 tonnes for fresh chili, and 320,000 tonnes for dry chili (FAOSTAT, 2020). Nevertheless, plant pathogenic fungi that caused a serious problem for chili production. For example, frogeye leaf spot (*Cercospora* sp.), wilt (*Fusarium* sp.), anthracnose (*Colletotrichum gloeosporioides*, *C. capsici* and *C. acutatum*), fruit and root rot (*Phytophthora capsici*) are the most insidious diseases that threaten chili crops and cause severe reductions in both

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quality and quantity (Majid *et al*., 2016). Growers have usually relied on chemical fungicides to manage various diseases due to their convenience of use. On the other hand, the disadvantage of chemical fungicides is their negative impact on ecosystems and danger to consumers. Nowadays, alternative applications such as plant extracts are increasingly being chosen to reduce or eliminate the toxicity of chemical fungicides, as the use of plant extracts has little to no negative impact on humans or the environment (Abou-Jawdah *et al*., 2004).

Gomphrena celosioides Mart. (Gomphrena weed) is a weed herb that belongs to the Amaranthaceae family of flowering plants (Siqueira, 1994). Growth of this weed in fields and unattended plots has caused problems for the production of main crops as it is difficult to control. However, the benefit of this weed in terms of medical and antimicrobial efficacy has been reported (Ogundipe *et al*., 2008; Tiwari *et al*., 2014). Furthermore, the extracts from this weed have been reported to inhibit the growth of *Aspergillus niger*, *Candida albicans*, and *Trichophyton* species (Dosumu *et al*., 2010; Tarnam *et al*., 2014). Nonetheless, few studies have focused on its phytochemical and bioactivity for use in controlling plant pathogenic fungi.

This study was conducted under laboratory conditions to evaluate the qualitative phytochemical properties and feasible antifungal potency of ethanolic crude extracts from Gomphrena weed for use in controlling phytopathogenic fungi which cause chili diseases.

Materials and methods

Plant preparation and extraction

The fresh Gomphrena weed used in the study was collected from Sakeao province, Thailand. The harvested plants were rinsed with tap water and separated into flowers and other aerial parts. The plants were dried in the open air for 7 days. The dried plants were ground with a blender. The powder of each plant part (400 g) was extracted with ethanol solvent (300 ml) using the Soxhlet extraction apparatus. A rotary evaporator was used to remove the ethanolic solvent of crude extracts. Then the ethanolic crude extracts of the Gomphrena weed were stored at 4 ℃ until use.

Phytochemical screening

The phytochemical components of the ethanolic crude extracts from Gomphrena weed were screened, and visual assessment was made for the

presence or absence of alkaloids, flavonoids, phenolics/tannins, saponins, and terpenoids using standard methods as follows:

Alkaloids (Dragendroff test): 15 ml of 2% H_2SO_4 was added to 0.2 g of the extract of each plant part. After that, Dragendroff's reagent was dropped into the extract solution. A red precipitate indicated the presence of alkaloids (Pandey and Tripathi, 2014).

Flavonoids (Shinoda test): 0.5 g of magnesium powder and 2 drops of HCl were added to 3 ml of distilled water mixed with the extract. The formation of red color indicated the presence of flavonoids (Hassan *et al*., 2004).

Saponins (Foam test): 0.5 g of each plant extract was mixed with 2 ml of distilled water and shaken. An observation of foam in the extract lasting ten minutes indicated the presence of saponins (Pandey and Tripathi, 2014).

Phenolics/tannins (Ferric chloride test): 0.2 g of extract in 1 ml of distilled water was added to 3-5 drops of ferric chloride solution. Bluish black precipitate indicated the presence of phenolics/tannins (Pandey and Tripathi, 2014).

Terpenoids: 0.2 g of each plant extract was dissolved with chloroform. 3 ml of H_2SO_4 was then added to the plant extract. A brown ring at the interface indicated the presence of terpenoids (Uddin *et al.*, 2011).

Fungal cultures and pathogenicity test

The pathogenic fungi, namely, *Cercospora* sp. (frogeye leaf spot), *Colletotrichum capsici, C. gloeosporioides* (fruit anthracnose), *Fusarium* sp. and *Sclerotium* sp. (root rot) were isolated from infected chili plants (Red Spur chile peppers) by tissue transplanting technique. Then, morphological identifications (Hussain and Abid, 2011; Than *et al*., 2008; Ferniah *et al*., 2014; Boukaew *et al*., 2011) were made on the collected pathogenic fungi. Subsequently, the pathogenicity of all isolated fungi was determined with detached leaf and fruit test by mycelial disc as well as seed germination test.

A mycelial disc (7 days old) of the fungi causing frogeye leaf spot and anthracnose symptoms were placed onto leaves and fruits, respectively. Then, all treatments were evaluated at 3, 5, 7 days and 3, 5, 7, 9, 11 days after inoculation, respectively, on the basis of lesion size on leaves and fruits. Disease severity was scored on a 0 to 9 scale, where 0 was on infection and 9 was infection greater than 25% (modified method of Montri *et al*., 2009). For the seed germination test, 5 ml of sclerotium or spore suspension (10^6 spores/ml) was soaked on 25 chili seeds. The tested seeds were placed on moist filter paper on a Petri plate, then all the plates were incubated at room temperature. This experiment was conducted in 4 replications. The percentage of infection was estimated from seed germination (modified method of Lazreg *et al*., 2014).

Antifungal activity of Gomphrena weed extracts

Preliminary test by paper disc diffusion technique

A preliminary test of antifungal activity of Gomphrena weed extract against the growth of 5 tested fungi was performed using the disc diffusion technique. 30 µl of plant extracts at each concentration of 80, 160 and 240 mg/ml were dripped onto paper discs and 2 control discs (1 mg/ml of benomyl and distilled water). A mycelial disc (7 days old) of 5 mm diameter was placed on the center of the fresh PDA plate. The above-prepared paper discs were placed around the mycelial disc at a distance of 2 cm. Then, all tested plates were incubated at 32 ℃ for 7 days. A completely randomized design (CRD) with 5 replications was used in the experiment. The mycelial growth was evaluated by measuring the radius of colonies (cm) and photographed (Foss *et al*., 2014).

Inhibition test by poisoned food technique

Mycelial growth test: The inhibition test of ethanolic extracts from the flowers and other aerial parts of Gomphrena weed to assess protection against the mycelial growth of 5 tested fungi was conducted using the poisoned food technique. Molten PDA was mixed with each plant extract and poured into a petri dish of 9 cm diameter. The final concentrations were 40, 80 and 160 mg/ml. Then, a mycelial agar plug of tested fungi (7 days old) was inoculated at the center of the prepared plate. Ten percent of Tween 20 and 1 mg/ml of benomyl were used as inoculated and chemical control treatments, respectively. All tested plates were incubated at 32 ℃ for 7 days. This experiment was designed in CRD with 3 replications. The antifungal activity was evaluated by measuring the diameter of mycelial growth in control and treatment plates. The inhibition of mycelial growth was calculated using the following equation: Percent inhibition = (Dc-Dt)/Dc × 100; where Dc was the diameter of mycelial growth of the control plate and Dt was the diameter of mycelial growth of the treatment plate.

Spore germination test: The effect of ethanolic extracts from the other aerial parts and flower of the Gomphrena weed on the spore germination of three tested fungi was estimated. The spore suspensions of *C. capsici*, *C. gloeosporioides*, and *Fusarium* sp. at 1×10^6 spores/ml were prepared. Then, 1 ml of the tested spore suspension was mixed with each plant extract. The final concentrations were 40, 80 and 160 mg/ml. Distilled water and benomyl were used as control treatments. The experiment was conducted in four replications and designed with CRD. Germination of spore was observed under a compound microscope at 12 and 24 h. The percentage of inhibition was calculated using

the formula: Percent inhibition = (Gc-Gt)/Gc ×100; Gc was the control, and Gt was germinated in treatment.

Results

Extract yield and phytochemical component

The obtained yield of ethanol crude extract of Gomphrena weed is shown in Table 1. Using 400 g of plant dry weight, the other aerial parts and flower had an extraction yield of 36 and 44 g, respectively. Regarding the phytochemical screening, the qualitative phytochemical of ethanolic crude extract of both parts of Gomphrena weed showed the presence of all tested components including alkaloids, flavonoids, phenolics/tannins, saponins, and terpenoids (Table 2).

Table 1. The yield of ethanolic crude extract of Gomphrena weed

Plant part	Fresh weight (g)	Dry weight (g)	Solvent volume (ml)	Extract yield (g)
other aerial parts	2002.32	400	300	36
flower	805.68	400	300	44

Table 2. Qualitative phytochemical screening of ethanol crude extract of Gomphrena weed

Phytochemical	Plant part	
	Other aerial parts	Flower
alkaloids	+[1/]	+
flavonoids	+	+
phenolics/tannins	+	+
saponins	+	+
terpenoids	+	+

[1/]+ = presence, - = absence

Fungal isolation, identification, and pathogenicity test

Cercospora sp., *Colletotrichum capsici*, *C. gloeosporioides*, *Fusarium* sp. and *Sclerotium* sp. were isolated from infected chili plant organs, and then identifications were made. The result presented disease symptoms, morphological characteristics of pathogenic fungi such as colony, hypha and conidia (Figure 1).

Regarding the frogeye leaf spot disease, its symptoms included necrotic, circular growth with a greyish white center encircled by a dark ring. Fungal colony on PDA: ash-gray to black zonated. Mycelia: branched, septate. Conidia located on light brown, unbranched conidiophores. These characteristics were identified as *Cercospora* sp. (Figure 1).

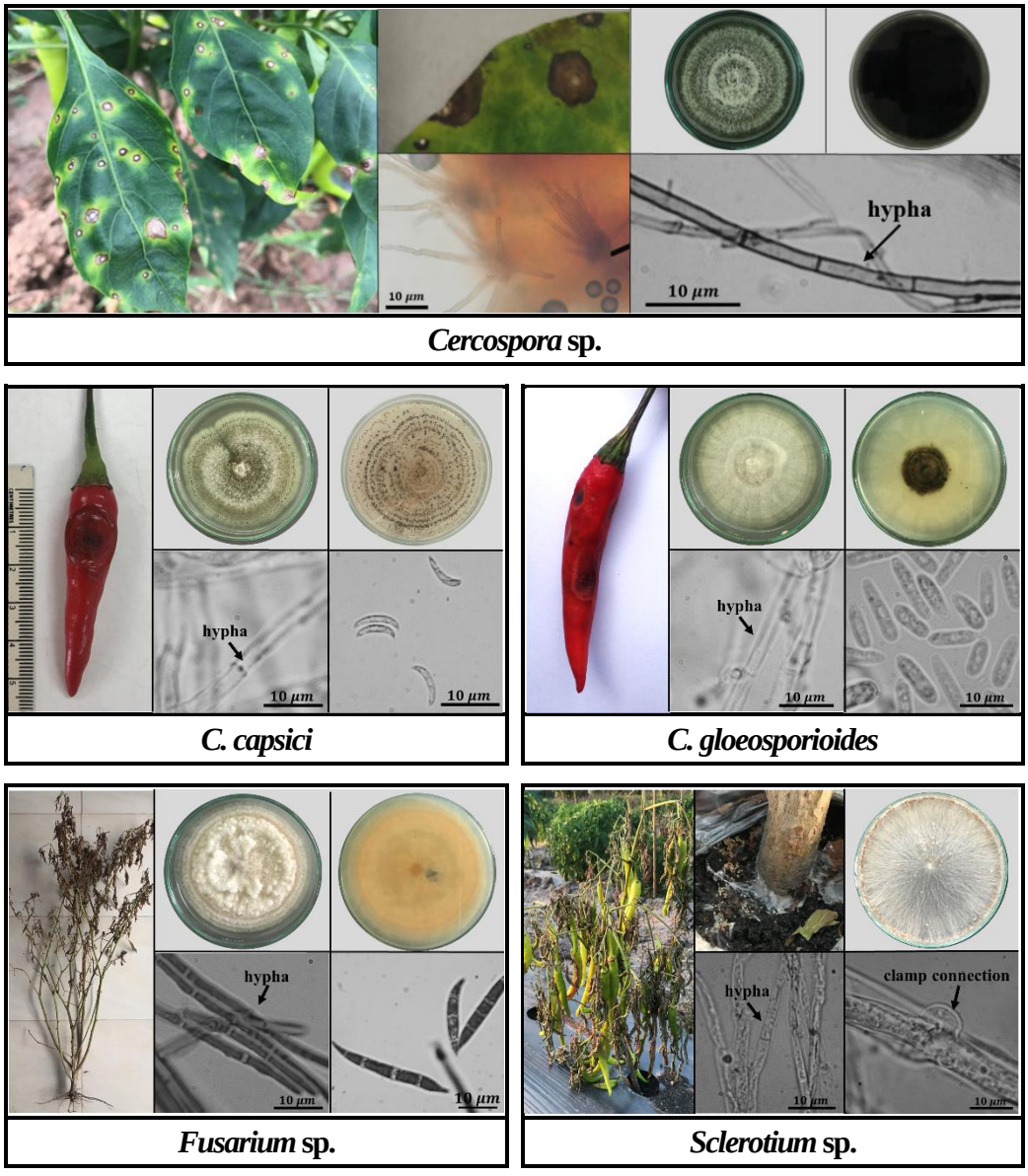


Figure 1. Symptoms and morphologies of plant pathogenic fungi causing chili diseases

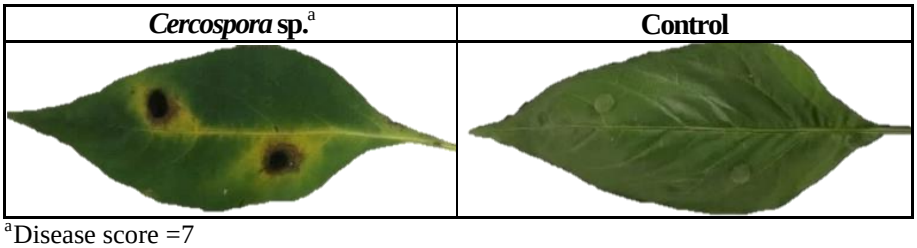
There were 2 types of colony characters and conidia morphologies: a) white colony, producing acervulus with nested circle and 1-cell conidia, hyaline, falcate shape, 1.5-3×8-10 µm and b) pale grey colony, and 1-cell conidia, hyaline,

cylindrical shape, 2-4.5×8-13.5 µm. The two isolates were identified as *C. capsici* and *C. gloeosporioides*, respectively (Figure 1).

Concerning the wilting leaf and browning stem of chili plants, the specified morphological characters were as follows: colony with a white cottony, under colony with whitish-yellow, and macroconidia with a falcate shape, hyaline, 3-5 septates and 3-4.5×20-40 µm. These characteristics were identified as *Fusarium* sp. (Figure 1).

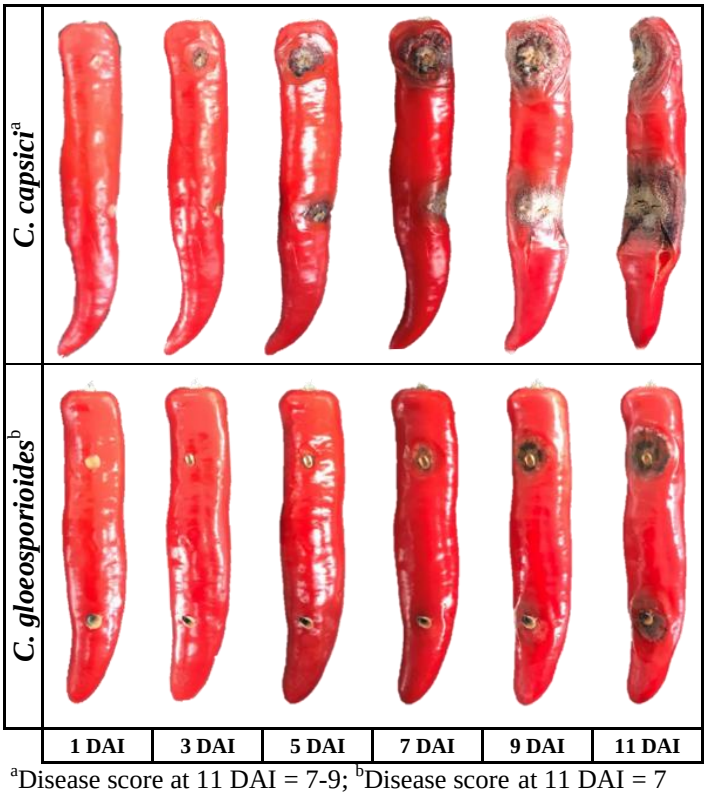
For the chili root wilt, a significant amount of white mycelia and dark brown sclerotia were found on the stem. The fungus produced a smooth colony and sclerotia after 12 days of incubation. Septate hypha was hyaline and clamp connections were noted. These morphological characteristics were identified as *Sclerotium* sp. (Figure 1).

The pathogenicity test on detached leaf assay (*Cercospora* sp.) and detached fruit assay (*C. capsici* and *C. gloeosporioides*) were established based on a disease severity score (0 to 9) with the percent lesion length relative to the overall length of leaf and fruit, respectively. The results revealed that the three above-mentioned fungi were the causal agents of chili diseases. *Cercospora* sp. caused leaf spot disease severity with the score of 7 while *C. capsici* and *C. gloeosporioides* gave disease severity of anthracnose with the score of 7-9 and 7, respectively (Figure 2 and Figure 3). Based on, the seed germination assay, *Fusarium* sp. and *Sclerotium* sp. were proven to be the causal agent of wilt and root rot of chili. *Fusarium* sp. reduced seed germination about 72 percent, while *Sclerotium* sp. completely inhibited seed germination 100 percent (Figure 4).



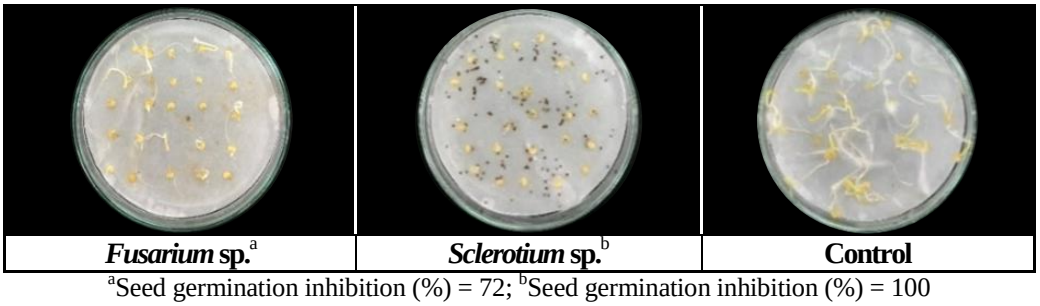
[a]Disease score =7

Figure 2. Pathogenicity test of *Cercospora* sp. on detached leaves of chili at 7 DAI



[a]Disease score at 11 DAI = 7-9; [b]Disease score at 11 DAI = 7

Figure 3. Pathogenicity test of *C. capsici* and *C. gloeosporioides* causing anthracnose disease on chili fruit



[a]Seed germination inhibition (%) = 72; [b]Seed germination inhibition (%) = 100

Figure 4. Pathogenicity test of *Fusarium* sp. and *Sclerotium* sp. using seed germination test at 9 DAI

Antifungal activity of Gomphrena weed extracts

Preliminary test by paper disc diffusion technique

The preliminary test using disc diffusion assay revealed that the Gomphrena weed extract demonstrated antifungal activity on some tested fungi. For the other aerial parts extract, the concentrations of 160 and 240 mg/ml significantly reduced the mycelial growth of *C. capsici*, *C. gloeosporioides* and *Fusarium* sp. compared to the control. Meanwhile, the 80 mg/ml concentration had an insignificant effect. Unexpectedly, no antifungal activity was detected either on *Cercospora* sp. or *Sclerotium* sp. The flower extract showed its antifungal potential at the concentrations of 160 and 240 mg/ml, which significant only on *C. gloeosporioides* (Table 3 and Figure 5).

Inhibition test by poisoned food technique

Mycelial growth test: The poisoned food assay revealed that the weed extract from both flowers and other aerial parts at all tested concentrations significantly inhibited the mycelial growth of all tested fungi except for *C. capsici* compared to the controls (water and benomyl). The growth inhibition appears to be corroborated with the extract concentrations, especially in the case of *C. gloeosporioides* and *Sclerotium* sp. Furthermore, the extract at the highest concentration (160 mg/ml) showed the greatest inhibitory effect on mycelial growth of *Sclerotium* sp. (100 percent) followed by *C. gloeosporioides* (82.5 percent), *Cercospora* sp. (41.76 percent) and *Fusarium* sp. (24.4 percent). Furthermore, it was observed that at all concentrations of the extract from the other aerial parts were most pronounced in inhibiting *C. gloeosporioides* in the ranges of 48.16-82.5 percent. Meanwhile, the extract from the flower part exhibited the greatest inhibitory effect against *Sclerotium* sp. in the ranges of 55.29-100 percent. Overall, the results obtained from this *in vitro* experiment indicated that the tested weed extract possessed substantial antifungal potency against mycelial growth of 4 tested fungi (Figure 6).

Spore germination test: The effect of the ethanolic crude extracts from the other aerial parts and flower at different concentrations (40, 80 and 160 mg/ml) was conducted against spore germination of *C. capsici*, *C. gloeosporioides,* and *Fusarium* sp. At 24 hr, it was revealed that the weed extracts from both plant parts at all concentrations were capable of complete inhibition of spore germination of all three tested fungi (Figure 4). Abnormalities of spores such as a combination of cytoplasm, swelling and lysis were noted (Figure 7).

Table 3. Effect of ethanolic crude extracts from Gomphrena weed on mycelial growth of plant pathogenic fungi causing chili diseases by paper disc diffusion technique

Plant part	Concentration	Radius of colony growth (cm)				
		Cercospora sp.	*C. capsici*	*C. gloeosporioides*	*Fusarium* sp.	*Sclerotium* sp.
other aerial parts	80 mg/ml	2.24a[1/]	2.08ab	2.01a	2ab	2.28a
	160 mg/ml	2.17a	2.05b	1.66b	1.92ab	2.16a
	240 mg/ml	2.11a	2.03b	1.37c	1.86b	2.1a
	Benomyl	2.18a	2.06ab	1.49bc	1.61c	2.18a
	Control	2.15a	2.28a	2.14a	2.12a	2.48a
flower	80 mg/ml	2.12a	2.12a	2.35ab	1.82ab	2.18a
	160 mg/ml	2.09a	2.15a	2.31b	1.74ab	2.1a
	240 mg/ml	2.05a	2.05a	2.29b	1.68ab	2a
	Benomyl	2.18a	2.08a	2.35ab	1.52b	2.22a
	Control	2.31a	2.2a	2.47a	1.96a	2.32a

[1/]Values are the average of five replications. Values in the same column within each plant part followed by the same letter are not significantly different as determined with Tukey HSK (P>0.05)

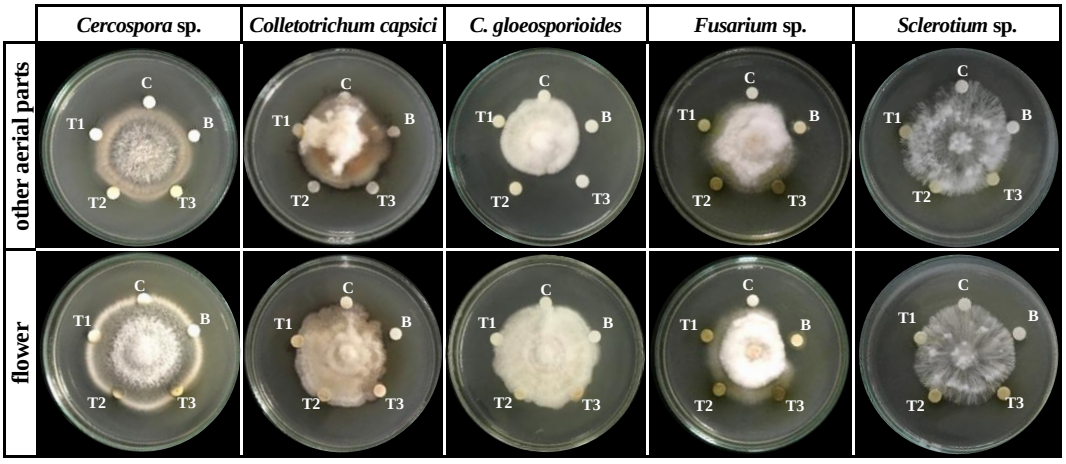


Figure 5. The colony of test fungi on paper disc diffusion assay of Gomphrena weed (T1 = 80 mg/ml, T2 = 160 mg/ml, T3 = 240 mg/ml, B = Benomyl and C = Control)

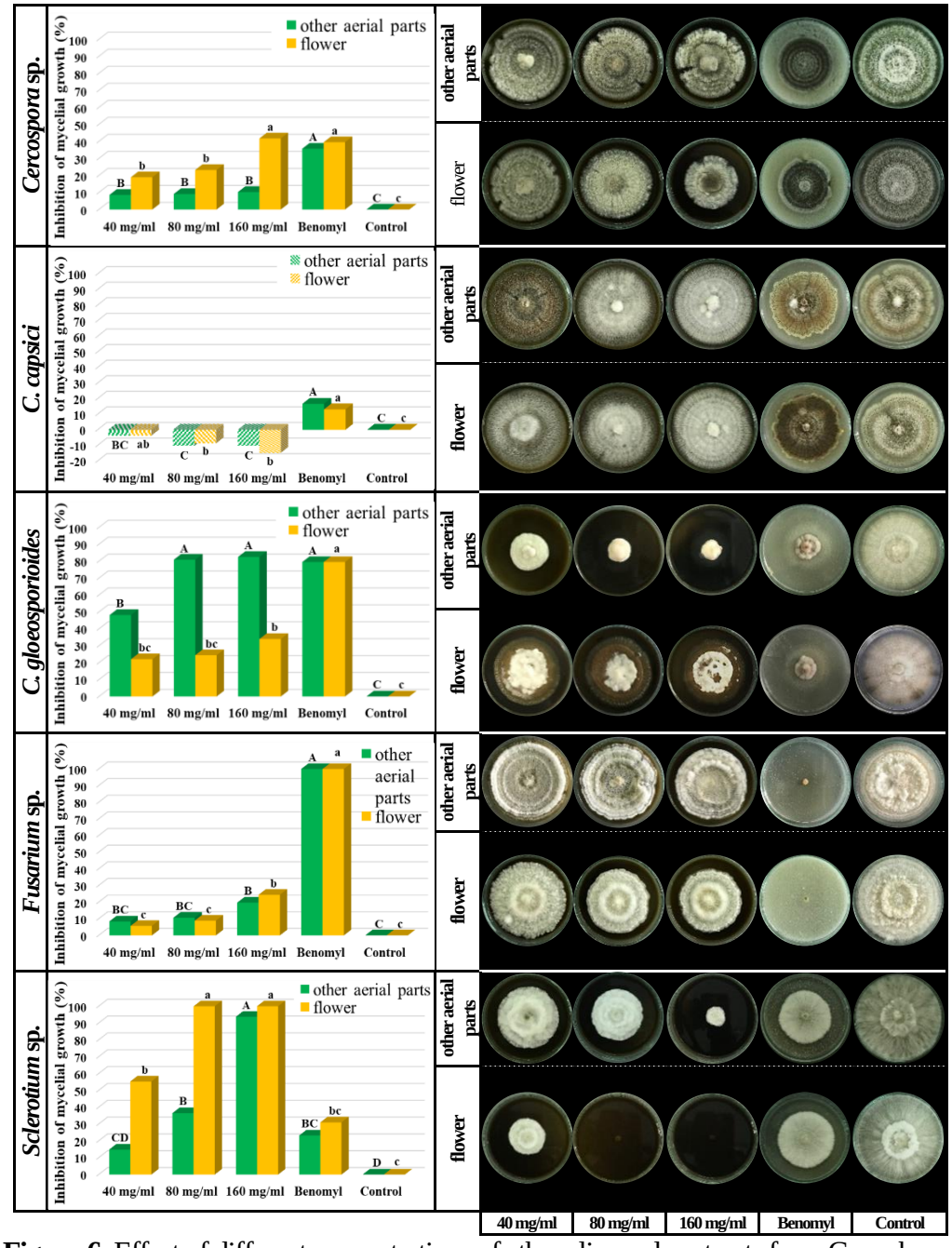


Figure 6. Effect of different concentrations of ethanolic crude extracts from Gomphrena weed on mycelial growth of plant pathogenic fungi by poisoned food technique (left); their characteristic colonies (right). The same capital letter on the green bar as well as the small letter on the yellow bar are not significantly different according to Tukey HSK (P>0.05)

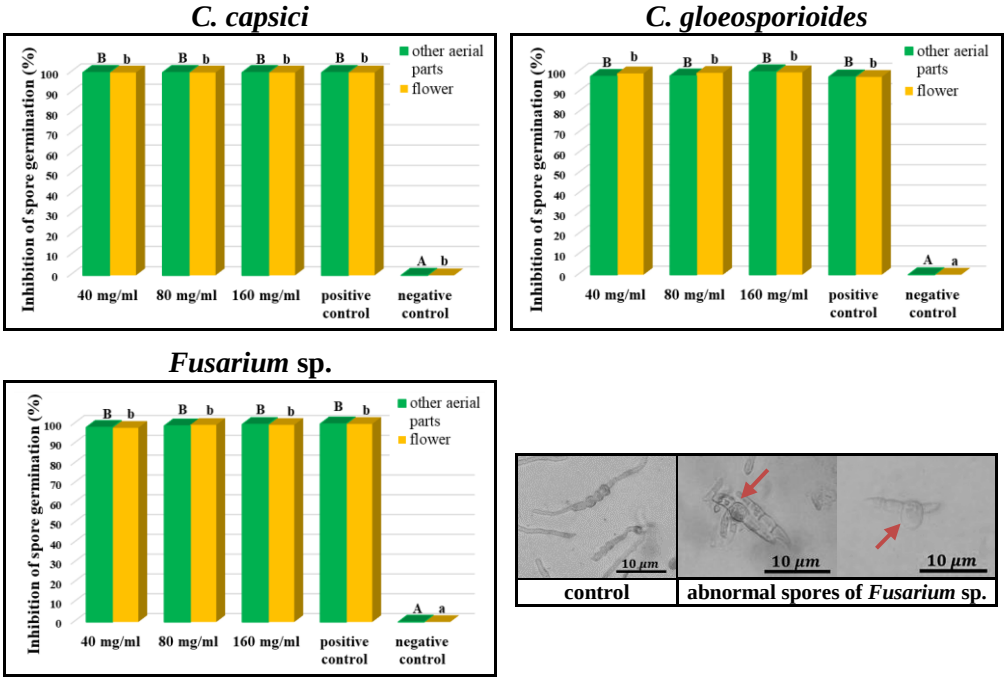


Figure 7. Inhibition effect of ethanolic crude extracts from Gomphrena weed on spore germination of plant pathogenic fungi at 24 h. The same capital letter on the green bar as well as the small letter on the yellow bar are not significantly different according to Tukey HSK (P>0.05)

Discussion

Gomphrena celosioides Mart. is well-known for its medicinal value worldwide. Therefore, our study was first conducted to evaluate the qualitative phytochemical compounds for the purpose of plant disease control. Accordingly, the active compounds, namely alkaloids, flavonoids, phenolics/tannins, saponins and terpenoids were identified in both flowers and other aerial parts of the extract. It was in agreement with the studies of Babu *et al*. (2012); Adeoti *et al*. (2016); Thuy *et al*. (2020); Ogundipe *et al*. (2008), who demonstrated the presence of the above-mentioned phytochemical compounds in the Gomphrena weed extract.

Five fungal isolates were isolated from the chili tissues showing disease symptoms such as frogeye leaf spot, anthracnose, stem browning and wilt. Subsequently, the collected isolates were identified by their morphological characteristics (Than *et al*., 2008; Boukaew *et al*., 2011; Hussain and Abid, 2011; Ferniah *et al*., 2014) as *Cercospora* sp., *Colletotrichum capsici*, *C. gloeosporioides* and *Sclerotium* sp. In addition, the isolates were pathogenicity

proven to confirm the level of their ability to cause disease. Our finding agreed with a number of other researchers (Than *et al*., 2008; Montri *et al*., 2009; Boukaew *et al*., 2011; Hussain and Abid, 2011; Ferniah *et al*., 2014; Mishra *et al*., 2018; Bijeeta *et al*., 2020) who reported that chili diseases caused by *Colletotrichum* spp., *Cercospora* sp., *Fusarium* spp., *Sclerotium* sp. *Rhizoctonia* spp. and *Phytophthora* sp. were important problems in chili production.

The effect of ethanolic crude extracts from the flowers and other aerial parts of Gomphrena weed in protecting against plant pathogenic fungi causing chili diseases was evaluated under laboratory conditions. Regarding the evaluation or screening the *in vitro* antifungal activity of the plant extract, the most known, basic and convenient methods such as disc diffusion, broth or agar dilution methods, as well as the poisoned food technique can be used (Balouiri *et al*., 2016). The disc diffusion method was employed in our preliminary test, and subsequently, the poisoned food technique.

In the preliminary test using the disc diffusion method, the extracts from both flowers and other aerial parts at 80, 160 and 240 mg/ml were shown to significantly inhibit the mycelial growth of only two tested fungi, namely *C. gloeosporioides* and *Fusarium* sp. Subsequently, the poisoned food experiment revealed the greatest inhibitory effect of the extract from both plant parts at the highest concentration (160 mg/ml) on mycelial growth of four tested fungi, namely *Sclerotium* sp. (100 percent), *C. gloeosporioides* (82.5 percent), *Cercospora* sp. (41.76 percent) and *Fusarium* sp. (24.41 percent). Based on the above-mentioned results, the weed extract seemed to exhibit greater potential against the tested fungi in the poisoned food experiment. This was probably due to differences in the reliability, efficacy and accuracy among the methods (Lehtopolku *et al*., 2012). However, the results from the two methods used appear to confirm the substantial antifungal potency of the weed extract.

Data regarding the substantial antifungal activities of the ethanolic Gomphrena weed extract obtained from our study were in line with other publications documenting the efficacy of the *Gomphrena celosioides* Mart. extracts in traditional medicine for protecting against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (Dosumu *et al*.; 2010; Sharma *et al*., 2011); *Bacillus subtilis*, *Salmonella typhi*, *Candida albicans*, *Aspergillus niger* and *Trichophyton* species (Dosumu *et al*., 2010). Moreover, remarkable antifungal protection against food-borne pathogens such as *Aspergillus flavus*, *A. niger*, *Penicillium ochrochloron* and *P. verrucosum* was noted from the pigmented extract of *Gomphrena globose* (Roriz *et al*., 2018). Additionally, our findings were in line with other studies that highlighted the plant members of the Family Amaranthaceae (e.g. *Amaranthus* spp., *Achyranthes fauriei*, *Mirabilis jalapa*) as plants having antifungal potency against a broad spectrum

of plant pathogens; *Colletotrichum lagenarium*: anthracnose in cucumber (Inagaki *et al*., 2008); *C. gloeosporioides*: anthracnose of black pepper (Biju and Praveena, 2018); *Fusarium verticillioides*: mycotoxin in maize (Thembo *et al*., 2010); *Alternaria alternata*, *Aspergillus flavus*, *Drechslera australiensis*, *Fusarium oxysporum* and *Macrophomina phaseolina* (Akbar *et al*. 2018; Amin *et al.,* 2021; Javaid *et al.,* 2017).

It was clearly seen that the Gomphrena weed extracts from both plant parts showed great response at all three concentrations against spore germination of the three tested fungi. This implies that a potential exists for a biofungicide from this weed to inhibit pathogen dissemination, infective capacity and progression in the host. These results are in agreement with the work of Bouhlali *et al*. (2021)

Surprisingly, it was found that not all fungal species were successfully inhibited by the weed extract, for instance *C. capsici*. On the contrary, the weed extract actually promoted the mycelial growth of *C. capsici*. Meanwhile, the extract was highly effective in inhibiting the spore germination of this fungus. This phenomenon noted in the study is in line with the work of Nduagu *et al*. (2008), which reported that leaf extracts from some plants, such as *Cochlospermum planchonii* (Hook F.) did not inhibit the colony diameter of *C. capsici*, but the stem bark extract completely inhibited sporulation.

Overall, our results provide strong evidence that the Gomphrena weed extract is highly effective in inhibiting mycelial growth and especially the spore germination of the tested fungi. Regarding this, the groups of phytochemical components present in the weed extract were likely to be responsible for the notable antifungal potency of the extract. This finding is in accordance with the work of Bouhlali *et al*. (2021), which demonstrated that the antifungal effect of the weed extracts could be attributed to their antioxidant polyphenols content and other antioxidants.

In conclusion, the study confirmed the presence of quality phytochemical compounds such as alkaloids, flavonoids, phenolics/tannins, saponins and terpenoids in the ethanolic crude extract of *Gomphrena celosioides* Mart. The findings demonstrate the notable antifungal activity of the ethanolic Gomphrena weed extracts in protecting against plant pathogenic fungi that cause chili diseases. Therefore, it is noteworthy that investigation of other weed species would be a valuable target in the search for effective natural products such as biofungicide to control plant diseases. For further study, the possible protective effect of this weed extract for protecting against *C. gloeosporioides* when applied in advance of pathogen inoculation would be of great interest.

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